A Novel OPA3 Mutation Revealed by Exome Sequencing

An Example of Reverse Phenotyping

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**Importance:** We sought to unravel the genetic cause in a consanguineous Pakistani family with a complex neurological phenotype.

**Observations:** Neurological and ophthalmological examination, including videotaping and fundoscopy, and genetic investigations, including homozygosity mapping and exome sequencing, were performed at the University of the Punjab and the University of Lübeck. Participants included 2 severely affected cousins from consanguineous parents, 10 of their reportedly unaffected relatives, and 342 Pakistani controls. Motor symptoms in the 2 patients started at the age of 3 to 4 years and included chorea, cerebellar ataxia, dystonia, and pyramidal tract signs. Genome-wide genotyping delineated 2 regions of homozygosity on chromosomes 13q12.11 to 13q12.13 and 19q12 to 19q13.41. Exome sequencing revealed 2 rare, homozygous variants (c.32

**Conclusions and Relevance:** Mutations in OPA3 have been reported in Costeff optic atrophy syndrome. We identify a novel missense mutation in OPA3 as the cause of a complex neurological disorder, expanding the OPA3-linked phenotype by early-onset pyramidal tract signs and marked lower limb dystonia. Investigation of optic atrophy was initiated only after genetic analysis, a phenomenon referred to as reverse phenotyping.

**Methods:**

Costeff optic atrophy syndrome (or optic atrophy plus syndrome; MIM 258501) is a rare autosomal recessive disorder originally described in the Iraqi-Jewish community in Israel. Patients typically develop optic atrophy and/or a choreoathetoid movement disorder within the first decade of life. Subsequent developments include spastic paraparesis, ataxia, and occasionally a mild cognitive deficit in the second decade of life.5,6

Costeff optic atrophy syndrome is caused by mutations in the optic atrophy 3 (OPA3) gene.3 This gene has 3 exons and is expressed in 2 transcripts, OPA3A (RefSeq NM_025136) and OPA3B (RefSeq NM_001017989). A homozygous splice site mutation (IVS1-1G>C) has been identified in OPA3 in all cases of Costeff optic atrophy syndrome in the Iraqi-Jewish population.3 Two additional homozygous mutations were found in a Turkish-Kurdish patient (c.320_337del [p.Q108_E113del]) and an Indian patient (c.415C>T [p.Q139X]). In addition, 2 heterozygous missense mutations, c.277G>A (p.G93S) and c.313C>G (p.Q105E), have been detected in patients with autosomal dominant optic atrophy and cataract (ADOAC [MIM 165300]).6

We performed homozygosity mapping and exome sequencing in a consanguineous Pakistani family affected by a complex neurological disorder. A novel OPA3 mutation was identified, prompting reevaluation of the clinical phenotype and the diagnosis of an optic atrophy plus syndrome.

The study was approved by the institutional review board at the University of the Punjab, and all participants gave written informed consent. Neurological examination was per-
formed by physicians in Pakistan (G.A.), and videotapes were reviewed by neurologists at the University of Lübeck (K.R.K. and C.K.). Healthy control subjects (who underwent screening for a history of movement disorders) were recruited from Pakistan (n=342). Fundoscopy in patients IV:1 and IV:6 was performed at the Department of Ophthalmology, Layton Rehmatullah Benevolent Trust.

Samples of DNA were available for 12 family members. Because dystonic and myoclonic features were present in the affected individuals, we first excluded mutations in the DYT1 gene by Sanger sequencing and linkage to DYT11 and DYT13 by microsatellite marker genotyping.

Genome-wide linkage analysis in 2 affected individuals and 4 unaffected members of the family DYAF09 was performed using a commercially available assay kit (GeneChip Human Mapping 250K Nsp Array; Affymetrix). Parametric linkage analysis was performed by the program Allegro® initially with a reduced marker panel of approximately 20,044 single-nucleotide polymorphisms. In regions with an LOD (logarithm [base 10] of odds) score greater than 2.5, all genotyped single-nucleotide polymorphisms were analyzed to map the homozygous regions precisely. Haplotypes were reconstructed with Allegro and presented graphically with HaploPainter. All data handling was performed using the graphical user interface ALOHOMORA.8

For exome sequencing, exonic regions were enriched using an exome library (Nimblegen SeqCap Human Exome, version 2.0; http://www.nimblegen.com/products/sequcap/ez/v2/index .html) and sequenced on a commercially available platform (Illumina HiSeq 2000; http://www.illumina.com/documents /products/datasheets/datasheet_hiseq2000.pdf) using paired-end reads of 2×100 base pairs (bp) at Atlas Biolabs, Berlin, Germany. The 30× coverage of the targeted regions was 89.2%, with a mean coverage of 91. Primary data were filtered, reads were mapped to the human reference genome (build 37), and variant calling was performed as described. Variants were filtered to (1) be rare, (2) affect a protein or have a location nearby a splice site (±15 bp), (3) be located in the linked regions, and (4) be homozygous (variant call, >75%). Verification included resequencing by Sanger sequencing and testing for segregation in all available family members. The frequency of segregating variants in ethnically matched controls was also determined by sequencing.

**RESULTS**

**CLINICAL FINDINGS**

Two individuals were severely affected (patients IV:1 and IV:6) in family DYAF09 (Figure 1A). Furthermore, the mother of patient IV:6 was observed to have upper limb dystonia.

Patient IV:1 had a normal birth and early development. At the age of 3 to 4 years, he started tiptoe walking. On examination at the age of 10 years, the patient had evidence of generalized dystonia predominantly affecting the lower limbs with marked foot inversion and plantar flexion leading to a pes equinovarus posture. He was able to mobilize with unilateral assistance. Subtle choreiform and myoclonic movements of the face, neck, shoulders, and arms were observed. Corticospinal tract signs in the legs (moderate spasticity, reflexes of +++, and a positive Babinski sign) were seen, but power was intact. No cerebellar signs were noted. At the age of 13 years, his gait showed deteriora-

Patient IV:6 had a normal birth and early development, but by the age of 5 to 6 years, her gait was unsteady with frequent falls. On examination at age 9 years, she had a broad-based, ataxic gait with dystonic posturing of the lower limbs. Choreiform and myoclonic movements of the trunk and limbs were observed. Evidence of appendicular ataxia was seen but no corticospinal tract signs.

In patients IV:1 and IV:6, ophthalmological assessment (postgenetic analysis) revealed symptoms of blurred vision, a bilateral reduction of visual acuity to counting fingers, and normal retinal reflexes and macular configuration. Bilateral advanced disc cupping, optic disc pallor, elongated narrow retinal vessels, and normal intraocular pressure were consistent with bilateral optic atrophy (Figure 1C). Extraocular eye movements were intact, and cognition appeared normal in both individuals. Sensory modalities were preserved, and results of cardiovascular, respiratory, and gastrointestinal tract examinations were normal. Cerebral magnetic resonance imaging and laboratory investigations including complete blood cell count and measurement of copper and ceruloplasmin levels were within reference limits for subject IV:1. Analysis of vitamin B12 levels showed them to be within reference limits in patient IV:6. Urinary organic acid screening could not be performed owing to the unavailability of testing facilities.

Individual III:1 (the mother of patient IV:6) showed evidence of bilateral hand dystonia (greater in the left than in the right hand; Figure 1D). She reported onset of this posture in adulthood. She was not available for detailed neurological or ophthalmological assessment.

**GENETIC INVESTIGATIONS**

Genome-wide linkage analysis revealed 2 homozygous regions on chromosomes 13q12.11 to 13q12.13 (size, 3.9 megabases [Mb], including 21 genes) and 19q12 to 19q13.41 (size, 24.3 Mb, including >400 genes) with a maximum LOD score of 2.71 (eFigure; http://www .jamanetwork.com). Using exome sequencing, 10 novel homozygous variants were identified in the linked regions (9 on chromosome 19 and 1 on chromosome 13) in a DNA sample from patient IV:6 (eTable). All 10 variants were confirmed by direct Sanger sequencing. Four variants in PARP4, FFA13, ZC3H4, and MED25 did not segregate with the disease. Four other variants (in MYBPC2, CLC, NPHS1, and CCDC61) were found in more than 3 Pakistani controls (frequency, >1%) and were thus considered unlikely to be disease causing.

The 2 remaining homozygous variants on chromosome 19 were both evolutionarily conserved and absent on the exome variant protein server (National Heart, Lung, and Blood Institute Exome Sequencing Project [http://evs.gs.washington.edu/EVS/]). The first variant was a missense change (c.941 C>G [p.A314G]) in the TSHZ3 gene (RefSeq NM_020856). This variant was found in 1 of the 342 Pakistani controls and was predicted to be benign by online programs Polyphen2 (Polyorphism Phenotyping, version 2; http://genetics.bwh
The p.L11Q mutation in OPA3 was identified as the most likely cause of the complex, heterogeneous neurological phenotype in family DYAF09. To our knowledge, this mutation is the fourth reported homozygous mutation and the first reported mutation to affect both transcripts of OPA3 (OPA3A and OPA3B; Figure 2). The mutation is also the first reported missense mutation in OPA3 causing autosomal recessive disease. The mutation is located within a potential mitochondrial leader sequence, implicating a pathogenic mechanism involving dysfunctional mitochondrial targeting of the OPA3 protein.

Although the TSHZ3 mutation also segregated with the disease, the OPA3 mutation is a much more convincing candidate because of its complete absence in controls and the presence of clinical features known to be associated with OPA3 mutations (eg, optic atrophy and choreoathetosis). However, a possible modifying role for the TSHZ3 mutation in this family cannot be excluded.
The patients exhibited many known clinical signs of Costeff optic atrophy syndrome, but some atypical phenotypic manifestations were also observed. For example, dystonia has not been previously reported in the context of Costeff optic atrophy syndrome, and spasticity and ataxia typically have a delayed onset in the second decade of life. Individual III:1, a heterozygous p.L11Q mutation carrier, showed evidence of adult-onset upper limb dystonia. However, whether this phenotype is related to the heterozygous OPA3 mutation remains unclear given that several other unaffected heterozygous mutation carriers were in the pedigree.

Exome sequencing is revolutionizing mendelian disease gene identification and may be used for improved clinical diagnosis. In this family, ophthalmological review obtained after genetic analysis revealed critical evidence of optic atrophy. Thus, exome sequencing identified a mutation in a gene known to cause disease, prompting the clinicians to reevaluate the phenotype and make the correct diagnosis, consistent with a phenomenon referred to as reverse phenotyping.

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